AR201-14084

Sarah_Loftus@americanchemistry.com on 11/26/2002 02:59:44 PM

To: Rtk Chem/DC/USEPA/US@EPA, oppt.ncic@epamail.epa.gov
cc:

Subject: HERTG HPV submission for polybutylene succinic anhydride

HPV Test Plan Submission from the American Chemistry Council Petroleum Additives HERTG - HPV Registration Number

Three documents (1. cover letter, 2. test plan and 3. robust summaries) are attached to this e-mail for the HERTG HPV Polybutylene Succinic Anhydrides group. If
you have any questions or comments, please feel free to contact me. Below, my contact information is listed. Thank you very much. Sarah McLallen

Sarah Loftus McLallen
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(See attached file: Test Plan.zip)

Test Plan.zip

November 26, 2002

By Mail
Christine Todd Whitman, Administrator
US EPA
PO Box 1473
Merrifield, VA 22116

Attn: Chemical Right-to-Know Program – Test Plan Submission from HERTG
Registration Number

Dear Administrator Whitman:

The American Chemistry Council Petroleum Additives Panel (Panel) Health, Environmental, and Regulatory Task Group (HERTG) submits for review and public comment its test plan report, as well as related robust summaries, for the *"Polybutylene Succinic Anhydries"* under the Environmental Protection Agency's High Production Volume (HPV) Chemical Challenge Program. The HERTG understands that there will be a 120-day review period for the test plan report and that all comments generated by or provided to EPA will be forwarded to the HERTG for consideration.

The polybutylene succinic anhydrides, which are used as petroleum lubricant additives, are characterized by having structural similarities and limited reactivity, low biological activity, and limited water solubility. Based upon the data reviewed in the attached report, the HERTG concludes that the physicochemical and toxicological properties of the proposed polybutylene succinic anhydrides group are similar and follow a regular pattern as a result of structural similarity. The two chemicals in the polybutylene succinic anhydrides group are as follows:

- 2,5-Furandione, dihydro-, monopolyisobutylene derivs., (CAS #67762-77-0), referred to as "Polyisobutylene succinic anhydride".
- 2,5-Furandione, dihydro-, monopolybutenyl derivs., (CAS #67762-79-2), referred to as "Polybutenyl succinic anhydride".

Briefly, the test plan for the HERTG polybutylene succinic anhydrides includes the following tests and computer modeling:

- Physicochemical - The water solubility of polyisobutylene succinic anhydride (CAS #67762-77-0) will be determined.

- Hydrolysis - The potential for polyisobutylene succinic anhydride (CAS #67762-77-0) to hydrolyze will be characterized. The public and available private literature will be evaluated to determine whether there is sufficient information to adequately characterize the potential hydrolysis rate of polyisobutylene succinic anhydride (CAS #67762-77-0). If it is determined that there is a lack of adequate information, this substance will be tested to develop hydrolytic rate data. If sufficient information is available in the general literature, it will be provided in the form of a robust summary.
- Photodegradation - The chemical structure of category members will be evaluated to determine whether there is a potential for direct photodegradation. Data will also be developed to characterize indirect photodegradation for category members using the AOP model in EPIWIN. Information or data for both routes of degradation will be provided in robust summaries.
- Fugacity modeling - Environmental partitioning data for members of this category will be calculated using a Mackay Level I equilibrium partitioning model and provided in robust summaries.
- Mutagenicity - *In vitro* chromosome aberration study will be conducted on the polyisobutylene succinic anhydride (CAS #67762-77-0). Results will be bridged to the other member of the category.
- Repeated-dose toxicity - A technical discussion document is proposed to address repeated-dose toxicity of members of the category based on read-across from test results of the structurally similar, *tetrapropenyl butanedioc acid (CAS # 27859-58-1)*.
- Reproductive/developmental toxicity - A technical discussion document is proposed to address reproductive/developmental toxicity of members of the category based on read-across from test results of the structurally similar, *tetrapropenyl butanedioc acid (CAS # 27859-58-1)*.

Thank you in advance for your attention to this matter. If you have any questions regarding the test plan report or the robust summaries, or HERTG's activities associated with the Challenge Program, please contact Sarah McLallen at 703-741-5607 (telephone), 703-741-6091 (telefax) or Sarah_McLallen@americanchemistry.com (e-mail).

Sincerely yours,

Courtney M. Price
Vice President, CHEMSTAR

cc: HERTG members

AR201-14084A

HIGH PRODUCTION VOLUME (HPV)

CHALLENGE PROGRAM

TEST PLAN

For

POLYBUTYLENE SUCCINIC ANHYDRIDES

Prepared by
The American Chemistry Council
Petroleum Additives Panel
Health, Environmental, and Regulatory Task Group

November 26, 2002

LIST OF MEMBER COMPANIES IN THE HEALTH, ENVIRONMENTAL, AND REGULATORY TASK GROUP

The Health, Environmental, and Regulatory Task Group (HERTG) of the American Chemistry Council Petroleum Additives Panel includes the following member companies:

B.P. PLC

Chevron Oronite Company, LLC

Crompton Corporation

Ethyl Corporation

ExxonMobil Chemical Company

Ferro Corporation

Infineum

The Lubrizol Corporation

Rhein Chemie Corporation

Rhodia, Inc.

EXECUTIVE SUMMARY

The American Chemistry Council Petroleum Additives Panel Health, Environmental, and Regulatory Task Group (HERTG), and its member companies, hereby submit for review and public comment their test plan for the *"Polybutylene Succinic Anhydride"* category of chemicals under the United States Environmental Protection Agency High Production Volume (HPV) Chemical Challenge Program. This report should be read in its entirety in order to obtain an understanding of the chemical category and proposed testing.

Polybutylene Succinic Anhydrides. Relying on several factors specified in the EPA guidance document on "Development of Chemical Categories in the HPV Challenge Program," in which use of chemical categories is encouraged, the following two closely related chemicals constitute a chemical category:

- 2,5-Furandione, dihydro-, monopolyisobutylene derivs., (CAS #67762-77-0), referred to as "Polyisobutylene succinic anhydride".
- 2,5-Furandione, dihydro-, monopolybutenyl derivs., (CAS #67762-79-2), referred to as "Polybutenyl succinic anhydride".

Structural Similarity. A key factor supporting the classification of these chemicals as a category is their structural similarity. Both substances in this category consist of a succinic anhydride structure with a polybutene substituent group.

Similarity of Physicochemical Properties. The similarity of the *physicochemical properties* of these substances parallels their structural similarity. All are dark colored viscous liquids intended for use as intermediates and components in finished lubricating oils. The use of these substances in finished lubricants requires that they be stable under high temperatures (>100^{o}C). Their low volatility is due to their low vapor pressure, high viscosity, and relatively high molecular weights of 500 to 2500 daltons. The existing information for these substances indicates that they have low water solubility (<10 mg/L based on calculated data). The polybutylene succinic anhydrides can undergo hydrolysis to butanedioic acid, polybutenyl derivatives.

Fate and Transport Characteristics. A member of this category, polyisobutylene succinic anhydride, has been shown to have limited biodegradability. Existing information for the anhydrides suggests that they will undergo hydrolysis and the hydrolyzed products (butanedioic acid, polybutenyl derivatives) will be the forms that should be considered when evaluating environmental fate. Direct photodegradation is not expected to cause significant physical degradation of members in this category. However, computer-modeled data will be developed to adequately characterize their potential to oxidize as a result of hydroxyl (OH-) radical attack. These substances are not expected to significantly partition to air if released into the environment because of their relatively low vapor pressure. Computer-modeled environmental partitioning data will be calculated for members of this category to adequately determine their potential to partition to other environmental compartments.

Toxicological Similarity. Review of reliable published and unpublished test data for members of the polybutylene succinic anhydride category suggests that the toxicity profiles of these chemicals are similar. Data obtained from proposed additional testing will further characterize the toxicological endpoints in the HPV Challenge Program for all members within this category.

Aquatic Toxicology. Data are available to adequately characterize fish, invertebrate, and alga toxicity**,** indicating a low concern for aquatic effects.

Mammalian Toxicology - Acute. Data on acute mammalian toxicity were reviewed, and the findings indicate a low concern for acute toxicity. Data are available for both members of the category indicating that the category has been well tested for acute mammalian effects. Therefore, no additional acute mammalian toxicity testing is necessary.

Mammalian Toxicology - Mutagenicity. Data from bacterial reverse mutation assays are available but not for *in vitro* chromosome aberration studies.

Mammalian Toxicology - Subchronic Toxicity. Data from repeated-dose toxicity studies are not available.

Mammalian Toxicology - Reproductive and Developmental Toxicity. Data from a reproductive/developmental toxicity screening study are not available.

Conclusion. Based upon the data reviewed for this test plan, the physicochemical, environmental fate, and toxicological properties of the proposed polybutylene succinic anhydride category members are similar and/or follow a predictable pattern based on structure similarity. Therefore, the EPA definition of a chemical category has been met, and the two chemicals that constitue the polybutylene succinic anhydride category will be tested in accordance with the test plan summarized below.

Test Plan. The test plan for the polybutylene succinic anhydride category includes the following testing, computer modeling, or technical discussion:

- Physicochemical - The water solubility of polyisobutylene succinic anhydride (CAS #67762-77-0) will be determined.
- Hydrolysis - The potential for polyisobutylene succinic anhydride (CAS #67762-77-0) to hydrolyze will be characterized. The public and available private literature will be evaluated to determine whether there is sufficient information to adequately characterize the potential hydrolysis rate of polyisobutylene succinic anhydride (CAS #67762-77-0). If it is determined that there is a lack of adequate information, this substance will be tested to develop hydrolytic rate data. If sufficient information is available in the general literature, it will be provided in the form of a robust summary.
- Photodegradation - The chemical structure of category members will be evaluated to determine whether there is a potential for direct photodegradation. Data will also be developed to characterize indirect photodegradation for category members using the AOP model in EPIWIN. Information or data for both routes of degradation will be provided in robust summaries.

- Fugacity modeling - Environmental partitioning data for members of this category will be calculated using a Mackay Level I equilibrium partitioning model and provided in robust summaries.
- Mutagenicity - *In vitro* chromosome aberration study will be conducted on the polyisobutylene succinic anhydride (CAS #67762-77-0). Results will be bridged to the other member of the category.
- Repeated-dose toxicity - A technical discussion document is proposed to address repeated-dose toxicity of members of the category based on read-across from test results of the structurally similar, *tetrapropenyl butanedioc acid (CAS # 27859-58-1*).
- Reproductive/developmental toxicity - A technical discussion document is proposed to address reproductive/developmental toxicity of members of the category based on read-across from test results of the structurally similar, *tetrapropenyl butanedioc acid (CAS # 27859-58-1)*.

As this test plan was developed, careful consideration was given to the number of animals that would be required for tests included in the proposed plan and conditions to which the animals might be exposed. In consideration of the concerns of some non-governmental organizations about animal welfare, the use of animals in this proposed test plan has been minimized.

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1.0 INTRODUCTION

In March 1999, the American Chemistry Council (formerly the Chemical Manufacturers Association) Petroleum Additives Panel Health, Environmental, and Regulatory Task Group (HERTG), and its participating member companies committed to address data needs for certain chemicals listed under the Environmental Protection Agency (EPA) High Production Volume (HPV) Chemical Challenge Program. This test plan follows up on that commitment.

Specifically, this test plan sets forth how the HERTG intends to address physico-chemical, environmental, aquatic and health effects testing information for the following two substances:

- 2,5-Furandione, dihydro-, monopolyisobutylene derivs., (CAS #67762-77-0), referred to as "Polyisobutylene succinic anhydride".
- 2,5-Furandione, dihydro-, monopolybutenyl derivs., (CAS #67762-79-2), referred to as "Polybutenyl succinic anhydride".

An analysis of the availble data on these chemicals supports the designation of the polybutylene succinic anhydride as a "chemical category" as provided in the EPA guidance document entitled, "Development of Chemical Categories in the HPV Challenge Program". This document provides the basis for the determination, indicates the findings of the data review process, and sets forth a proposed testing plan to satisfy parts of the required test battery for endpoints without data that would be considered adequate under the program.

EPA guidance on the HPV Chemical Challenge Program indicates that the primary purpose of the program is to encourage "the chemical industry . . . to voluntarily compile a Screening Information Data Set (SIDS) on all chemicals on the US HPV list." (EPA, "Development of Chemical Categories in the HPV Challenge Program," p. 1) At the same time, EPA recognizes that the "large number of chemicals to be tested [about 2800 HPV chemicals] makes it important to reduce the number of tests to be conducted, *where this is scientifically justifiable.*" (*Id.*, p. 1) [emphasis added] The next part of the guidance explains where this would be scientifically justifiable:

> One approach is to test closely related chemicals as a group, or category, rather than test them as individual chemicals. In the category approach, *not every chemical needs to be tested for every SIDS endpoint.* However, *the test data finally compiled* for the category must prove adequate to support a screening level hazard-assessment of the category and its members. That is, the *final data set* must allow one to estimate the hazard for the untested endpoints, *ideally* by interpolation between and among the category members. In certain cases, where toxicity is low and no upward trend is expected, extrapolation to the higher category members may be acceptable. (*Id.*, p. 1) [emphasis added].

EPA guidance goes on to state, "The use of categories is encouraged in the Challenge Program and will have a number of benefits." (*Id.,* p. 1) Among the benefits identified in the guidance for the use of categories are "a reduction in testing will result in fewer animals used to test a category of chemicals as opposed to doing each test on each individual chemical," and "there will be . . . economic savings since less testing may be needed for chemicals considered as a

category." (*Id.*, p. 1) That guidance also states that categories "accomplish the goal of the Challenge Program – to obtain screening level hazard information – through the strategic application of testing to the category." (*Id*, p. 2)

A similarly stated intent "to reduce the number of tests to be conducted, *where this is scientifically justifiable*" was articulated by the Agency in its draft guidance document titled, "The Use of Structure Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program." [emphasis added].

The EPA "Chemical Categories" guidance sets forth a definition of what constitutes a "chemical category, for the purposes of the Challenge Program". Specifically, that definition states that a chemical category under the HPV Challenge Program "is a group of chemicals whose physicochemical and toxicological properties *are likely to* be similar *or* follow a regular pattern as a result of structural similarity." (*Op. Cit*., p. 2) [emphasis added].

According to the guidance, what is important is that the "structural similarities [among members of the group] *may* create a predictable pattern *in any* or all of the following parameters: physicochemical properties, environmental fate and effects, and human health effects." (Id., p. 2) [emphasis added]. Thus, it is not necessary for the chemicals in a category to be similar in all respects. Nor must there be conclusive proof that the chemicals in the postulated category will behave identically across all relevant parameters. All that is required for an acceptable category under the HPV Challenge Program is that there be a *likelihood* of similarity of physicochemical and toxicological properties or a *likelihood* that the chemicals will in some pertinent respect follow a regular pattern as a result of their structural similarity.

In identifying the polybutylene succinic anhydride category, the six-step process set out in the EPA guidance on category development was followed. The polybutylene succinic anhydride chemicals clearly satisfy the guidance regarding use of a chemical category.

Step 1: group structurally similar chemicals into a putative category

Step 2: gather relevant published and unpublished literature for each member of the category

Step 3: evaluate the compiled data for adequacy in accordance with the EPA guidance documentation

Step 4: construct matrices of SIDS endpoints versus category members arranged so as to indicate the structural progression of the category (in this case, by increasing molecular weight)

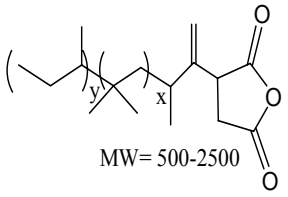
Step 5: evaluate the data to determine whether there is a correlation between category members for each SIDS endpoint

Step 6: make available to EPA, and to the public for review, this test plan including the foregoing category definition and rationale and the following data assessment with the proposed testing scheme for the polybutylene succinic anhydrides.

2.0 CHEMISTRY OF POLYBUTYLENE SUCCINIC ANHYDRIDES

2.1 DESCRIPTION

Polybutylene succinic anhydrides are amphiphilic molecules, wherein the hydrophobic polybutene is attached chemically to a hydrophilic succinic anhydride group. A general structure is shown below:



67762-79-2

The chemical names and CAS numbers for the members of the polybutylene succinic anhydride category are presented in Table 1 and the chemical structures in Table 2.

These substances are produced from the reaction of polybutenes and maleic anhydride to give polybutenyl succinic anhydride (PIBSA). At the end of the reaction, the unreacted maleic anhydride is stripped out of the mixture containing polybutenyl succinic anhydride, but the unreacted polybutene remains in the mixture. Polybutylene succinic anhydrides are either used as components or as intermediates in the synthesis of corrosion inhibitor components and succinimide dispersants for application in lubricants (e.g., motor oils, metalworking oils, industrial oils). Succinimides are made by reacting the intermediate PIBSA with polyethyleneamines. Other derivatives of PIBSA are used as stabilizers in emulsion explosives for mining. The diacid is made by adding water to the polybutylene succinic anhydride and is used as corrosion inhibitors.

2.2 PHYSICOCHEMICAL PROPERTIES

Selected physicochemical properties of members from the polybutylene succinic anhydride category are presented in Table 3.

2.2.1 Molecular Weight

Members of the category have molecular weights ranging from 500 to 2500 daltons (Table 3).

2.2.2 Specific Gravity

The specific gravity of category members is approximately 0.93 g/ml (@60/60°F) (Table 3).

2.2.3 Melting Point and Boiling Point

Polybutylene succinic anhydrides, as manufactured, are liquid at most ambient temperatures. Modeling data indicate that the melting point of these substances is approximately 188°C - 349 °C (Table 3). Modeling data indicate that the boiling point of these substances is approximately 476 - 1695 °C (Table 3).

2.2.4 Vapor Pressure and Viscosity

The low volatility of category members is associated with their low vapor pressure, high viscosity, and relative high molecular weights. Modeling data indicate that the vapor pressures of the members in category are equal to or less than 2.07e-009 Pa @ 25 °C (Table 3). The viscosity of polyisobutylene succinic anhydride (CAS #67762-77-0) derived from 1300 molecular weight polybutene is measured as 130 cSt @ 100°C (Table 3).

2.2.5 Water Solubility

The polybutylene succinic anhydrides contain a functional group that has the potential to hydrolyze. Polyisobutenyl butanedioic acid (CAS #68610-89-9) is the hydrolysis product of this anhydride. Modeling data indicate that the water solubility of the polyisobutenyl butanedioic acid is approximately 6.9e-009 mg/L to 0.0 (Table 3). This value indicates that the diacid of the anhydride members of this category are sparingly soluble to insoluble in water. This value will be confirmed by developing measured water solubility data for this substance.

2.2.6 Octanol-Water Partition Coefficient

Modeling data indicate the log octanol-water partition coefficient (Kow) value of the polyisobutenyl butanedioic acid (CAS #68610-89-9) to range from 13.08 to 77.95 (Table 3). Kow values for the anhydrides are not provided because these substances would not be present in their anhydride forms in the aqueous phase.

3.0 USES OF POLYBUTYLENE SUCCINIC ANHYDRIDES

Polybutylene succinic anhydrides are derived from the reaction of polybutene with maleic anhydride. They are commonly used as either components or as intermediates in the manufacture of oil soluble succinimide dispersants for lubricants, anti-rust additives, and emulsifiers. The finished lubricating oils include all types of internal combustion engine oils (e.g., automotive and diesel engine crankcase oils, air and water-cooled two-cycle engine oils, natural gas engine oils, marine trunk piston engine oils, medium-speed railroad diesel engine oils), automatic transmission fluids, and gear oils. The succinimide dispersants are used as ashless dispersant intermediates to inhibit colloidal particle-to-particle aggregation by an adsorbed film mechanism. They also are able to solubilize oil-insoluble liquids. Polybutylene succinic anhydride dispersant components are generally sold to finished oil blenders in additive packages, where the concentration ranges typically from 5 to 50 wt.%. These additive packages are then blended into finished oils where the typical concentration of polybutylene succinic anhydride dispersant ranges from 0.5 to 10 wt.% in the finished oil depending on the application.

Polybutylene succinic anhydride dispersants in this category are manufactured and blended into additive packages at plants owned by members of the HERTG. Finished lubricants are blended at facilities owned by HERTG's. Additive packages are shipped to customers in bulk in ships, isocontainers, railroad tank cars, tank trucks or in 55-gallon steel drums. The bulk additive packages are stored in bulk storage tanks at the customer blending sites. Finished oils are blended by pumping the lubricating oil blend stocks and the additive package from their storage tanks through computer controlled valves that meter the precise delivery of the components into a blending tank. After blending, the finished lubricant products are sold in bulk and shipped in tank trucks to large industrial users, such as manufacturing facilities and facilities that service truck fleets and passenger motor vehicles. Finished lubricants are also packaged into 55-gallon drums, 5-gallon pails, and one-gallon and one-quart containers for sale to smaller industrial users. Sales of lubricants in one-gallon and one-quart containers to consumers at service stations or retail specialty stores also occur.

Based on these uses, the potentially exposed populations include (1) workers involved in the manufacture of polybutylene succinic anhydride dispersants, blending them into additive packages, and blending the additive packages into finished lubricants; (2) quality assurance workers who sample and analyze these products to ensure that they meet specifications; (3) workers involved in the transfer and transport of polybutylene succinic anhydride dispersants, additive packages or finished lubricants that contain them; (4) mechanics who may come into contact with both fresh and used lubricants while working on engines or equipment; (5) gasoline station attendants and consumers who may periodically add lubricating oil to automotive crankcases; and (6) consumers who may change their own automotive engine oil. The most likely route of exposure for these substances is skin and eye contact. Manufacturing, quality

assurance, and transportation workers will likely have access to engineering controls and wear protective clothing to eliminate exposure. Mechanics wear protective clothing, but often work without gloves or eye protection. Gasoline station attendants and consumers often work without gloves or other protective equipment. The most likely source of environmental exposure is accidental spills at manufacturing sites and during transport.

4.0 EVALUATION OF AVAILABLE PUBLIC AND COMPANY DATA

4.1 Environmental Fate Data

4.1.1 Physicochemical Properties Relevant to Environmental Fate

In order to evaluate the environmental fate of a substance, it is important to understand its potential degradability and partitioning behavior among environmental compartments (i.e., air, soil, sediment, suspended sediment, water, and biota).

The physicochemical properties and molecular structure of a chemical will influence the degradation processes it may be subjected to in the environment. Potentially important environmental degradation pathways include biodegradation, hydrolysis, and photodegradation. Biodegradation of an organic chemical by bacteria can provide energy and carbon for microbial growth. This process results in a structural change of the chemical. Biodegradation can result in the complete loss of an organic chemical, producing carbon dioxide, mineral salts, and water. Hydrolysis is a reaction in which a water molecule or hydroxide ion substitutes for another atom or group of atoms present in an organic chemical resulting in a structural change of that chemical. Chemical photodegradation results in a structural change of a molecule from the absorption of solar radiation.

The physicochemical properties of a substance will also influence the way in which it partitions among environmental compartments. Generally, substances characterized by a low vapor pressure do not partition into air to any great extent. Similarly, substances that are characterized by a lower water solubility do not partition extensively into water. Substances that do not partition into air and water to any great extent tend to partition into soil and sediments.

4.1.2 Biodegradation

4.1.2.1 Test Methodologies

The potential biodegradability of a substance in water, under aerobic conditions can be assessed using one of the OECD 301 testing guidelines. Chemical biodegradation involves a series of microbial-mediated reactions that can require many kinds of microorganisms acting together to degrade the parent substance.

There are several standard test methods, which measure primary degradation (i.e., loss of parent chemical) or ultimate degradation (i.e., complete utilization of the substance to produce carbon dioxide, water, mineral salts, and microbial biomass). Primary degradation can be determined analytically by measuring dissolved organic carbon (DOC) for water-soluble chemicals, infrared absorbance, or by a chemical-specific detection method. Ultimate degradation (also called mineralization) can be determined by measuring oxygen consumption or carbon dioxide evolution relative to the theoretical levels that can be achieved based on an elemental analysis of the chemical under investigation.

4.1.2.2 Summary of Available Data

Biodegradation data for the polybutylene succinic anhydride category are summarized in Table 4. One member of the category has been adequately tested.

The Modified Sturm Test (OECD Guideline 301B, *CO_2 Evolution Test*) was used to evaluate the biodegradability of polyisobutylene succinic anhydride (CAS # 67762-77-0). After the 28-day test, the extent of biodegradation was 2.3 to 5.0% based on carbon dioxide evolution.

4.1.2.3 Data Assessment and Test Plan for Biodegradability

Adequate biodegradation data exist for polyisobutylene succinic anhydride (CAS #67762-77-0). Since the alkyl side chains of substances in this category are predominantly branched, the results indicate that these substances would exhibit limited biodegradation under the conditions of the test system. These results will be used to bridge to the other member of the category, thereby characterizing the biodegradability of the entire category. No additional biodegradability testing is proposed.

4.1.3 Hydrolysis

4.1.3.1 Test Methodologies

The potential for a substance to hydrolyze in water can be assessed as a function of pH (OECD Guideline 111, *Hydrolysis as a Function of pH*[1]). When an organic molecule undergoes hydrolysis, a nucleophile (water or hydroxide ion) attacks an electrophile and displaces a leaving group (e.g., halogen, phenoxide)[2]. Potentially hydrolyzable groups include alkyl halides, amides, carbamates, carboxylic acid

[1] Organization for Economic Cooperation and Development (OECD) (1993) OECD Guidelines for Testing of Chemicals. OECD. Paris, France.
[2] W. Lyman et al. (1990) Handbook of Chemical Property Estimation Methods. McGraw-Hill Book Co. New York, NY, USA.

esters and lactones, epoxides, phosphate esters, and sulfonic acid esters[3], as well as anhydrides. Otherwise, the lack of a suitable leaving group renders compounds resistant to hydrolysis.

4.1.3.2 Summary of Available Data

There are no published or unpublished hydrolysis studies for members of this category.

4.1.3.3 Data Assessment and Test Plan for Hydrolysis

Polybutylene succinic anhydrides contain a functional group that has the potential to hydrolyze. Polybutylene butanedioic acid is the hydrolysis product of this anhydride. This reaction is believed to occur at a rapid rate. The public and private literature will be reviewed to identify if there is sufficient information that can be used to assess the potential hydrolysis rate of the polybutylene succinic anhydride. In the event that there is insufficient information, the hydrolysis rate of polyisobutylene succinic anhydride (CAS # 67762-77-0) will be evaluated using the testing method described above. Data will then be bridged to the other member of the category.

4.1.4 Photodegradation

4.1.4.1 Testing and Modeling Methodologies

Photodegradation can occur as a result of direct and indirect mechanisms. Direct photodegradation can be measured in solution using the OECD test guideline 113, while indirect photodegradation can be estimated using a model accepted by the US EPA.

Simple chemical structures can also be examined to determine whether a chemical has the potential for direct photolysis in water. First order reaction rates can be calculated for some chemicals that have a potential for direct photolysis using the procedures of Zepp and Cline[4].

An estimation method for indirect photodegradation that is accepted by the US EPA applies a calculation procedure to determine an atmospheric oxidation potential (AOP) value. The computer program AOPWIN (atmospheric oxidation program for Microsoft Windows) (EPIWIN, 1999) is used by the US EPA OPPTS (Office of Pollution Prevention and Toxic Substances) to estimate AOP values.

[3] W. Lyman et al. (1990) Handbook of Chemical Property Estimation Methods. McGraw-Hill Book Co. New York, NY, USA.

[4] Zepp, R. G., and D. M. Cline. 1977. Rates of Direct Photolysis in the Aqueous Environment. Environ. *Sci. Technol.* 11:359.366.

This program calculates a chemical half-life based on an overall OH- reaction rate constant, a 12-hr day, and a given OH- concentration.

4.1.4.2 Summary of Available Data

Published or unpublished photodegradation studies and AOP data for members of the polybutylene succinic anhydride category are not available.

4.1.4.3 Data Assessment and Test Plan for Photodegradation

Direct photochemical degradation occurs through the absorbance of solar radiation by a chemical substance. If the absorbed energy is high enough, then the resultant excited state of the chemical may lead to its transformation. A prerequisite for direct photodegradation is the ability of one or more bonds within a chemical to absorb ultraviolet (UV)/visible light in the 290 to 750 nm range. Light wavelengths longer than 750 nm do not contain sufficient energy to break chemical bonds, and wavelengths below 290 nm are shielded from the earth by the stratospheric ozone layer. Indirect photodegradation also requires light energy as well as a series of chemical reactions that include a reaction of the parent molecule with hydroxyl radicals (OH-).

An initial review of the members of the polybutylene succinic anhydride category suggests that the members do not contain bonds that have a high potential to absorb UV light above 290 nm. Further, these substances have low vapor pressure, which indicates that they have a low potential to partition into the air to a significant extent where they would be subject to indirect photodegradation.

To develop adequate data for this endpoint, the UV light absorptive potential of chemicals in this category will be evaluated to identify those chemicals with a potential to degrade in solution. When possible, first order reaction rates will be calculated for chemicals identified to have a potential for direct photolysis in water. The results of the calculations will be summarized in a technical discussion in the form of a robust summary. If instead, a low potential for direct photolysis is suggested by the evaluation, a technical discussion will be prepared as a robust summary describing the findings.

The AOP data for representative structures of the category (Table 2) will be estimated and the following data provided in a robust summary:

- Rate constants for the atmospheric, gas phase reaction as mediated by photochemically produced hydroxyl radicals.
- Atmospheric half-lives based on hydroxyl radical attack.

4.1.5 Fugacity Modeling

4.1.5.1 Modeling Methodologies

Fugacity-based multimedia fate modeling calculates the relative distribution of a chemical between environmental compartments. A widely used model for this approach is the EQC model[5].

There are multiple levels of the EQC model, which vary in complexity and data requirements. In the document, "Determining the Adequacy of Existing Data", EPA states that it accepts Level I fugacity modeling to estimate transport/distribution values. The EQC Level I model utilizes input of basic chemical properties, including molecular weight, vapor pressure, and water solubility to calculate percent distribution within a standardized environment (unit world). Another EQC model, the Level III model, uses these parameters, as well as chemical emission rates into air, water, and soil, and chemical degradation rates in air, water, soil, and sediment. Because much of this information is not available and because using default values could develop incorrect data, Level I partitioning data will be developed for members of this category.

4.1.5.2 Summary of Available Data

There are no published or unpublished fugacity-based multimedia fate modeling data for members of the polybutylene succinic anhydride category. All of the members of this category have low vapor pressure and are sparingly water soluble

suggesting that they will not tend to partition into the air or water to any great extent.

4.1.5.3 Test Plan for Fugacity

The relative distribution of substances within this category among environmental compartments will be evaluated using the Level I model. Data developed using a Level I model can then be used for simple comparative purposes across several substances. EQC Level III will not be used for this evaluation because appropriate emission levels are as yet unknown. Because of the physical nature of the substances in this category and the lack of emission data, a Level I data set will be as equally robust as a Level III data set and can then be used to assess the potential partitioning behavior of the category members in the environment.

Input data to run the EQC Level I model may require an additional computer model to estimate selected physical/chemical properties from a structure. The

[5] Mackay, D., A. Di Guardo, S. Paterson, and C. E. Cowan. 1996. Evaluating the Environmental Fate of a Variety of Types of Chemicals Using the EQC Model. Environ. Tox. Chem. 15:1627-1637.

model used for this purpose will be EPIWIN, version 3.04[6], which was developed by the Syracuse Research Corporation. EPIWIN includes algorithms for estimating all physical and chemical properties needed for the EQC model. The representative structures that will be used are listed in Table 2.

4.2. ECOTOXICOLOGY DATA

4.2.1 Aquatic Ecotoxicity Testing

4.2.1.1 Test Methodologies

Acute aquatic ecotoxicity testing can include three species that represent three tropic levels in the freshwater aquatic environment: fish, invertebrates, and algae. The fish acute toxicity test (OECD Guideline 203, *Fish, Acute Toxicity Test*) determines the lethality of a substance to a fish during a 96-hour exposure period. The invertebrate acute toxicity test (OECD Guideline 202, *Daphnia sp., Acute Immobilization Test and Reproduction Test*) determines the potential of a substance to immobilize an invertebrate, typically a daphnid (*Daphnia magna*), during a 48-hour exposure period. The alga growth inhibition test (OECD Guideline 201, *Alga, Growth Inhibition Test*) determines the potential of a substance to inhibit alga growth, typically using the freshwater unicellular green algae, *Pseudokirchneriella subcapitata* (formerly called *Selenastrum capricornutum*), during a 72- or 96-hour exposure period.

Three different exposure methodologies are available to conduct aquatic toxicity tests; i.e., flow-through, static, and static renewal.

In *flow-through exposures*, organisms are exposed to a constant chemical concentration or loading in each treatment level in the incoming water and there is typically greater assurance than with other test methods that the exposure levels and water quality remains constant throughout the test. Although flow-through testing is the preferred method, it is most applicable for chemicals that have adequate water solubility for testing.

In *static exposures*, organisms are exposed to a chemical in a test medium that is not replaced for the duration of the study. There is less assurance that the test concentrations or loadings to which test organisms are exposed will remain constant because test substance can be adsorbed onto test chambers, degraded, volatilized, or otherwise changed during the test. Nevertheless, due to limitations of other test systems for non-volatile substances, the static test has been widely used and in some instances must be used, as is the case when conducting an alga test.

[6] EPIWIN. 1999. Estimation Program Interface for Windows, version 3.04. Syracuse Research Corporation, Syracuse, NY, USA.

The *static-renewal exposure* is similar to a static exposure because it is conducted in still water, but the test solutions and control water are renewed periodically, usually every 24 hours. Daily test solution renewal provides a greater likelihood that the exposure concentrations or loadings will remain stable throughout the test. This is the preferred exposure method for conducting fish toxicity tests for compounds in this category. Daily renewals cannot be performed in the alga test because the process of exposure solution separation and replenishment can cause a discontinuity in the alga growth rate. Also, dependent on the substance and test procedure used, renewals may not be possible for the *Daphnia* test because the procedure can stress *Daphnia* or result in coating or entrapping the organisms in surface film that may form during renewal operations. OECD considers the use of static testing for fish, *Daphnia*, and algae, and the use of static renewal testing for fish to be appropriate when evaluating the toxicity of sparingly water-soluble substances like those in this category provided that test solution preparation uses water accommodated fraction or water soluble fraction methods.[7]

4.2.1.2 Test Solution Preparation

Polybutylene succinic anhydrides are sparingly soluble to insoluble in water, and it is not possible to prepare exposure solutions for aquatic toxicity testing by direct addition of measured quantities of test material to water. Two methods[8] are used to prepare solutions of poorly water-soluble materials for aquatic toxicity testing:

- *Water accommodated fraction* (WAF) – This is a method in which the test solution contains only that fraction of the test material (organic phase) which is retained in the aqueous phase after a period of stirring long enough to reach equilibrium, followed by a sufficient time (1-4 hours) for phase separation. The WAF (aqueous phase) will contain soluble components of the test material at levels that will be dependent on the test material loading (the amount of material added to the aqueous medium). The resulting WAF is used in the aquatic toxicity test. Ideally, a WAF consists of a water-soluble extract of test material, but it can also include a stable micro-emulsion or contain small amounts of suspended matter.

- *Water soluble fraction* (WSF) – This is a method in which a WAF is either filtered, centrifuged, or allowed to settle for a greater length of time (24 hours) than with the WAF method to remove suspended matter from the aqueous phase before being used in the aquatic toxicity test.

[7] Organization for Economic Cooperation and Development (OECD) (2000). Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures. OECD Environmental Health and Safety Publications, Series on Testing and Assessment No.23, Paris, France.

[8] American Society for Testing and Materials (1998) D6081-98, Standard Practice for Aquatic Toxicity Testing of Lubricants: Sample Preparation and Results Interpretation.

4.2.1.3 Reporting Toxicity Results

In both WAF and WSF tests, test material concentrations are expressed as loading rates (i.e., defined as the weight of test material added per unit volume of test medium during WAF or WSF preparation)[9]. For fish tests, endpoints can be expressed as median lethal loading rate (LL_{50}) when lethal effects occur to 50% of the test population or in cases where no lethal effects are observed at all loadings tested, LL_0. In both cases, results can be expressed in mg/L and in studies where no lethality is observed, the result is expressed as LL_0 = the highest loading rate tested. For invertebrate and alga tests, endpoints are expressed as median effective loading rate (EL_{50}) or EL_0 in mg/L as discussed above.

Loading rates allow sparingly water-soluble to insoluble complex substances such as the polybutylene succinic anhydrides to be compared to more readily soluble substances and/or pure chemicals on an equal basis. To allow comparison, the toxicity value is expressed as the amount of test material added per unit volume of water when preparing the WAF or WSF.

If test material exposure levels are analytically measured in the test, the endpoints can also be expressed as median lethal concentration (LC_{50}) or median effective concentration (EC_{50}) in mg/L. EC/LC_{50}s are often not reported because it is very difficult to accurately measure test material exposure levels that can be below 1.0 mg/L.

NOTE: In this test plan, these results are reported as loading rates (EL/LL), to reflect the current reporting practices for the WAF method used in the tests. In the robust summaries, these data are presented as concentrations (EC/LC) as originally reported even though the test methods employed WAF preparation of test solutions without measurement of test material concentration.

4.2.2 Aquatic Toxicity of Members from the Polybutylene Succinic Anhydride Category

In general, the toxicity of a substance to an organism is limited by mechanisms of uptake and movement to target organs. Characteristics such as smaller molecular size and a lesser degree of ionization increase the ability of a substance to passively cross biological membranes. However, the soluble fraction of a compound in water represents the chemical fraction responsible for toxicity to aquatic organisms. Therefore, aquatic toxicity can be limited by the water solubility of a substance.

Data and preliminary modeling information indicates that the members of the polybutylene succinic anhydride category have sparingly low water solubility.

[9] Organization for Economic Cooperation and Development (OECD) (1999) Draft Guidance document on Aquatic Toxicity Testing of Difficult Substances. OECD, France.

This assessment is based on the diacid form of the anhydrides. The diacid form is used because aquatic organisms will only be exposed to the diacid, which is the hydrolyzed form of the anhydride. The length of the polybutylene side chain on these substances will influence their relative water solubility, and thus possibly, their relative toxicity.

4.2.2.1 Summary of Available Data

Acute aquatic ecotoxicity data for the polybutylene succinic anhydride category is summarized in Table 5. One member of the category has been tested for acute aquatic toxicity in fish and invertebrates and for effects on algal growth. No toxicity was observed on the substance.

4.2.2.1.1 Fish Acute Toxicity

Polyisobutylene succinic anhydride (CAS #67762-77-0) was evaluated for acute toxicity to fish. The maximum test material loading rate was 1,000 mg/L. Mortality of 10 to 25% was observed at loading rates greater than 600 mg/L after 96 hours, and a loading rate-response relationship was apparent. The LL_{50} for this substance was greater than 1,000 mg/L, indicating a low order of toxicity to fish.

4.2.2.1.2 Invertebrate Acute Toxicity

Polyisobutylene succinic anhydride (CAS #67762-77-0) was evaluated for acute toxicity to daphnids (*Daphnia magna*). The single (maximum) test material loading rate was 1,000 mg/L. No mortality of *D. magna* occurred within the 48-hour exposure. The EL_{50} for this substance was greater than 1,000 mg/L, and the EL_0 determined in the test was 1,000 mg/L, indicating a low order of toxicity to aquatic invertebrates.

4.2.2.1.3 Alga Toxicity

Polyisobutylene succinic anhydride (CAS #67762-77-0) was evaluated for effects on the growth of the unicellular green alga, *Pseudokirchneriella subcapitata*, in a 96-hour exposure. The single (maximum) test substance loading rate was 1,000 mg/L. No inhibition of algal cell density was observed. The EL_{50} for this substance was greater than 1,000 mg/L.

4.2.2.2 Data Assessment and Test Plan for Acute Aquatic Ecotoxicity

Adequate acute aquatic ecotoxicity studies have been conducted for the polybutylene succinic anhydride category. These studies involved three trophic levels of aquatic organisms and evaluated the acute aquatic ecotoxicity of one of the two members of the category. The data demonstrate a low order of acute aquatic ecotoxicity, and can be used for bridging to the remaining category

member (polybutenyl succinic anhydride, CAS #67762-79-2). No further aquatic ecotoxicity testing is proposed.

4.3 MAMMALIAN TOXICOLOGY DATA

4.3.1 Physicochemical Properties Relevant to Mammalian Toxicity

Physicochemical properties of chemicals are useful for predicting the routes by which exposure may occur, and in some cases, the mechanism and extent of toxicological responses. The physicochemical properties of the polybutylene succinic anhydride are presented in Table 3. These lubricant additive intermediates are relatively high molecular weight, liquid substances with moderately high octanol/water partition coefficients and sparingly water solubilities. These characteristics indicate that the polybutylene succinic anhydride are slightly lipophilic, and thus, capable of passive diffusion across biological membranes. It would be predicted that upon oral exposure these chemical substances would be absorbed by the gastrointestinal tract. However, the structural and physical properties such as comparatively high molecular weight, the presence of long-chain polybutene moieties and sparingly water solubility is expected to impede the rate and extent of absorption of polybutylene succinic anhydride from dermal surfaces. In addition to the general considerations discussed above, the polybutylene succinic anhydrides have relatively high boiling points, low vapor pressure, and are viscous liquids. As a result, these substances have a low propensity to form vapors or aerosols, and thus, unintentional exposure via inhalation is uncommon.

4.3.2 Acute Mammalian Toxicity of Members from the Polybutylene Succinic Anhydride Category

4.3.2.1 Acute Toxicity Test Methodology

Acute toxicity studies investigate the effect(s) of a single exposure to a relatively high dose of a substance. Potential routes of exposure for acute toxicity assays include oral, dermal, and inhalation. Oral toxicity assays are conducted by administering test material to fasted animals (typically rats or mice) in a single gavage dose. Acute dermal toxicity tests are conducted by administering test material to the shaved skin on the back of the test animal (typically rats or rabbits) and allowing the test material to stay in contact with the skin application site for a specific duration (usually 24 hours). Acute inhalation toxicity assays are conducted by exposing test animals (typically rats) in a controlled atmosphere to a fixed air concentration of the test substance for a specific duration (typically 4 hours). The test material is either generated as a vapor or intentionally aerosolized into respirable particles, then metered into the exposure air at the desired concentration. Preferably, inhalation toxicity studies are conducted using either

nose-only or head-only exposure to minimize potential confounding effects resulting from whole-body exposure. Whole body exposure may lead to over-prediction of inhalation toxicity hazard by increasing the body-burden of the test material through skin absorption or ingestion of test material as a consequence of grooming both during and after the inhalation exposure period.

Historically, lethality is a primary end-point of concern in acute toxicity studies, and the traditional index of oral and dermal potency is the median lethal dose that causes mortality in 50 percent of the test animals (LD_{50}). In acute inhalation studies, the traditional measurement of potency is the median lethal concentration of the test material in air that causes mortality in 50 percent of the test animals (LC_{50}). In addition to lethality, acute toxicity studies also provide insights regarding potential systemic toxicity through careful observation and recording of clinical signs and symptoms of toxicity as well as through detailed examination of tissues and organ systems.

Typically, acute oral and dermal toxicity studies are conducted using a limit dose of 5000 and 2000 mg/kg body weight, respectively, and acute inhalation toxicity studies are conducted using a limit dose of 5 mg/L for 4 hours (according to OECD and EPA testing guidelines). Prior to 1990, some acute dermal toxicity studies may have used a limit dose of 5000 mg/kg. Recently, harmonized EPA testing guidelines (August 1998) have set the limit dose for both oral and dermal acute toxicity studies at 2000 mg/kg body weight, while the recommended limit concentration for acute inhalation studies has been set at 2 mg/L for 4 hours. The limit dose test method minimizes the number of animals tested by exposing a single group of animals to a large dose (the limit dose) of the test substance. A test substance that shows little or no effects at the limit dose is considered essentially nontoxic, and no further testing is needed. If compound-related mortality is observed at the limit dose, then further testing may be necessary.

4.3.2.2 Summary of Available Data

Acute toxicity data for the polybutylene succinic anhydride category is summarized in Table 6. Both members of the category have been tested by the oral and dermal route of administration and demonstrated a low order of acute toxicity.

4.3.2.2.1 Acute Oral Toxicity

The two substances in the polybutylene succinic anhydride category have been adequately tested for acute oral toxicity. The acute oral LD_{50} for these studies in rats is greater than 2000 mg/kg indicative of a relatively low order of acute toxicity.

4.3.2.2.2 Acute Dermal Toxicity

The two substances in the polybutylene succinic anhydride category have been adequately tested for acute dermal toxicity. The acute dermal LD_{50} for this study in rabbits was greater than 2000 mg/kg indicative of a relatively low order of acute toxicity.

4.3.2.3 Data Assessment and Test Plan for Acute Mammalian Toxicity

In total, four adequate acute toxicity studies have been conducted for members of the polybutylene succinic anhydride category. These studies involved two species of laboratory animals (rats or rabbits); two routes of exposure (oral and dermal); and evaluated the toxicity of the members of the category. The data consistently demonstrate a low order of acute toxicity. The overall low order of acute toxicity for these substances in combination with their similar chemical structure and physicochemical properties supports the scientific justification of these three substances as a category within the HPV Challenge Program.

Based on the results of these studies, the acute toxicity of the category has been evaluated adequately with respect to all acute toxicity endpoints, and no additional acute toxicity testing is proposed for the HPV Challenge Program**.**

4.3.3 Mutagenicity of Members from the Polybutylene Succinic Anhydride Category

4.3.3.1 Mutagenicity Test Methodology

Genetic toxicology is concerned with the effects of substances on genetic material (i.e., DNA and chromosomes). Within genetic material, the gene is the simplest functional unit composed of DNA. Mutations are generally non-lethal, heritable changes to genes that may arise spontaneously or because of xenobiotic exposure. Genetic mutations are commonly measured in bacterial and mammalian cells. The simplest test systems measure the occurrence of a base-pair substitution mutation in which a single nucleotide is changed followed by a subsequent change in the complementary nucleotide on the other DNA strand. Frame shift mutations occur following the deletion or insertion of one or more nucleotides, which then changes the "reading frame" for the remainder of the gene or multiple genes. Genetic testing for these types of point mutations is generally accomplished by *in vitro* cellular assays for forward or reverse mutations. A forward mutation occurs when there is a detectable change in native DNA whereas a reverse mutation occurs when a mutated cell is returned to its initial phenotype. Both base-pair substitutions and frame shift mutations are routinely measured in bacterial cells by measuring the ability of a cell to acquire the capability to grow in an environment missing an essential amino acid. In these tests, a large number of

cells are examined to demonstrate a significant increase in the frequencies of mutations that occur over the frequency of spontaneous mutations.

Chromosomal aberrations are large scale numerical or structural alterations in eukaryotic chromosomes including deletions (visualized as breaks), translocations (exchanges), non-disjunction (aneuploidy), and mitotic recombination. Chromosomal breakage is the classical end point in chromosomal aberration assays. Substances that induce structural changes in chromosomes, especially chromosome breaks, are referred to as "clastogens." To visualize chromosomes and chromosomal aberrations following *in vitro* or *in vivo* treatment with a substance, cells are arrested in metaphase, treated to swell the chromosomes, fixed, transferred to slides and stained. The first metaphase following treatment is the time at which the greatest number of cells with damaged chromosomes may be observed. The most frequently used test systems investigate changes in mammalian cells (such as Chinese hamster ovary or lung cells; human or rat lymphocytes; or human, rat or mouse bone marrow cells) following either *in vitro* or *in vivo* exposure to the test substance.

4.3.3.2 Summary of Mutagenicity Data

A summary of the mutagenicity information for the polybutylene succinic anhydride category is presented in Table 7. *In vitro* bacterial gene mutation assays have been conducted for members of the category. Frequencies of reverse mutations in bacteria were not significantly changed after exposure to members of the polybutylene succinic anhydride category. *In vitro* chromosomal aberration assays have not been conducted for the members of this category.

4.3.3.2.1 Bacterial Gene Mutation Assay

Both substances in this category have been adequately tested in a bacterial reverse mutation test (OECD Guidelines 471 and/or 472). Both tested substances were negative for mutagenic activity, with and without metabolic activation.

4.3.3.2.2 *In Vitro* Chromosomal Aberration Assays

The two substances in this category have not been adequately tested in an *in vitro* chromosomal aberration assay (OECD Guideline 473).

4.3.3.3 Data Assessment and Test Plan for Mutagenicity

The two members of the polybutylene succinic anhydride category have been adequately tested for mutagenicity in the gene mutation assay but not for chromosomal aberrations.

Polybutylene succinic anhydride (CAS #67762-77-0) will be tested and the data bridged to the other member in the category which lacks *in vitro* chromosomal aberration data for the HPV Challenge Program**.**

4.3.4 Repeated-dose Toxicity of Polybutylene Succinic Anhydride Category

4.3.4.1 Repeated-dose Toxicity Test Methodology

Repeated-dose toxicity studies evaluate the systemic effects of repeated exposure to a chemical over a significant period of the life span of an animal (rats, rabbits, or mice). Chronic repeated-dose toxicity studies are concerned with potential adverse effects upon exposure over the greater part of an organism's life span (e.g., one to two years in rodents). Subchronic repeated-dose studies are also concerned with effects caused by exposure for an extended period, but not one that constitutes a significant portion of the expected life span. Subchronic studies are useful in identifying target organ(s), and they can be used in selecting dose levels for longer-term studies. Typically, the exposure regimen in a subchronic study involves daily exposure (at least 5 consecutive days per week) for a period of at least 28 days or up to 90 days (i.e., 4 to 13 weeks). A recovery period of two to four weeks (generally included in most study designs) following completion of the dosing or exposure period provides information on whether or not the effects seen during the exposure period are reversible upon cessation of treatment. The dose levels evaluated in repeated-dose toxicity studies are notably lower than the relatively high limit doses used in acute toxicity studies. The NOAEL (no observed adverse effect level), usually expressed in mg/kg/day, defines the dose of test material that produces no significant toxicological effects. If the test material produces toxicity at the lowest dose tested (i.e., there is no defined NOAEL), the lowest dose that produced an adverse effect is defined as the LOAEL (lowest observed adverse effect level). While these studies are designed to assess systemic toxicity, the study protocol can be modified to incorporate evaluation of potential adverse reproductive and/or developmental effects.

Reproductive and developmental toxicity studies generate information on the effects of a test substance on male and female reproductive performance such as gonadal function, mating behavior, conception, and development of the conceptus, parturition, and post-partum development of the offspring. Various study designs exist, but they all involve exposure to both male and female test animals before mating. The rat is most often selected as the test species. The test substance is administered to males and females continuously at several graduated doses for at least two weeks prior to mating and until the animals are sacrificed. The males are treated for at least two more weeks. Male gonadal histopathology is carefully assessed at the end of the study. The females are treated through parturition and early lactation. The adult females and offspring are typically studied until termination on post-natal day 21, or sometimes earlier. In addition to providing data on fertility and reproduction, this study design provides information on potential developmental toxicity following prenatal and limited post-natal exposure to the test substance. An NOAEL or LOAEL is also used to describe the results of these tests, with the exception that these values are derived from effects specific to reproduction or development.

The "toxicity to reproduction" requirement in the HPV Challenge Program can be met by conducting the *Reproduction/Developmental Toxicity Screening Test* (OECD Guideline 421) or by adding this screening test to a repeated-dose study (OECD Guideline 422, *Combined Repeated Dose Toxicity Study with the Reproductive/Developmental Toxicity Screening Test*). The *One-Generation Reproduction Toxicity Study* (OECD Guideline 415) is a more comprehensive protocol for the study of the effect of a test material on reproduction and development that also meets the OECD SIDS and the HPV Challenge Program requirements.

4.3.4.2 Summary of Repeated-Dose Toxicity Data

None of the members from the polybutylene succinic anhydride category have been tested for repeated-dose or reproductive and development toxicity.

4.3.4.3 Data Assessment and Test Plan for Repeated-Dose Toxicity and Reproductive/Developmental Toxicity

The two members of the polybutylene succinic anhydride category have not been adequately tested for repeated-dose or reproductive/developmental toxicity. Based on the propensity of anhydrides to hydrolyze under aqueous conditions, the hydrolyzed polybutenyl butanedioic acid derivative is expected to have repeated-dose toxicity and reproductive/developmental effects comparable to the structurally similar tetrapropenyl butanedioic acid (CAS #27859-58-1). Tetrapropenyl butanedioic acid is a member of the HPV Challenge Program, Alkenyl Succinic Anhydride Category, that is proposed for repeated-dose and reproductive/developmental toxicity evaluation. The Repeated-dose and reproductive/developmental toxicity data from testing tetrapropenyl butanedioic acid will provide sufficient data for read-across assessment of the two members of the polybutylene succinic anhydride category due to their chemical structural similarities. Therefore, a technical discussion document is proposed to address repeated-dose and reproductive/developmental toxicity of polybutylene succinic anhydrides with no additional testing for the HPV Challenge Program.

Table 1. Members of the Polybutylene Succinic Anhydride Category

CAS Number	Chemical Name	Simplified Chemical Name
67762-77-0	2,5-Furandione, dihydro-, monopolyisobutylene derivatives	Polyisobutylene succinic anhydride
67762-79-2	2,5-Furandione, dihydro-, monopolybutenyl derivatives	Polybutenyl succinic anhydride

TABLE 2. Chemical Structures of Polybutylene Succinic Anhydride Category

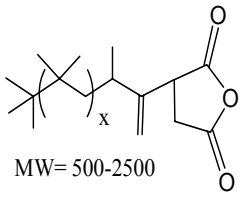
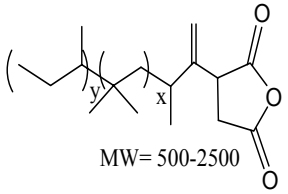
CAS Number	Chemical Structure
67762-77-0	 67762-77-0
67762-79-2	 67762-79-2

Table 3. Selected Physicochemical Properties and Proposed Testing of the Memebers of the Polybutylene Succinic Anhydride Category

CAS Number	Molecular Weight	Specific Gravity[1] (g/ml)	Viscosity[2] (cSt @ 100°C)	Melting Point[3] (°C)	Boiling Point[4] (°C)	Vapor Pressure[5] (Pa)	Water Solubility[11] (mg/L)	Log Kow
67762-77-0	518.87 to	~0.917	~130	188.46	476.62	2.07e-009	NA[7]	NA[8]
	2482.67	ND[12]	ND	349.84	1695.4	0.0	0.0	NA[8]
67762-79-2	500 to	~0.927	~140	ND	ND	3e-4	NA[9]	NA[10]
	2500	ND	ND	ND	ND	ND	NA[9]	NA[10]
68610-89-9 [6]	536.89 to	ND	ND	236.4	550.95	7.21e-012	6.884e-009	13.08
	2500.68	ND	ND	349.84	1769.7	0.0	0.0	77.95

[1] ASTM D4052, Standard Test Method for Density, Relative Density (Specific Gravity), or API Gravity of Crude Petroleum and Liquid Petroleum Products by Hydrometer Method.
[2] ASTM D 445-97, Standard Test Method for Kinematic Viscosity of Transparent and Opaque Liquids (the Calculation of Dynamic Viscosity).
[3] Modeling data; melting point is estimated (MPBWIN v1.40) and cannot be measured due to viscosity of liquid.
[4] Modeling data; boiling point is estimated (MPBWIN v1.40) and cannot be measured because these substances decompose before they boil.
[5] Modeling data; vapor pressure is estimated (MPBPWIN v1.40).
[6] Represents diacid of CAS #67762-77-0.
[7] Not applicable; anhydrides form diacids in aqueous solutions, the water solubility of the diacid of CAS #67762-77-0[6] is calculated to be 1.671e-01 mg/L to 0.0.
[8] Not applicable; anhydrides form diacids in aqueous solutions, the Log Kow of the diacid is calculated to be 14.65 to 77.95.
[9] Not applicable; anhydrides form diacids in aqueous solutions.
[10] Not applicable; anhydrides form diacids in aqueous solutions.
[11] Modeling data; water solubility is estimated (KOWWIN v1.65).
[12] ND = Not determined.

Table 4. Environmental Fate Data and Proposed Testing for Members of the Polybutylene Succinic Anhydrides Category

CAS Number	BIODEGRADATION	HYDROLYSIS	PHOTODEGRADATION	FUGACITY
	Available Data & Proposed Testing	**Available Data & Proposed Testing**	**Available Data & Proposed Testing**	**Available Data & Proposed Testing**
67762-77-0	**2.5 - 5.0% biodegraded after 28-days**	Literature review or testing if needed	Direct photodegradation evaluation AOPWIN model estimation	EQC model estimation
67762-79-2	No testing needed Bridging	Literature review	Direct photodegradation evaluation AOPWIN model estimation	EQC model estimation

Table 5. Aquatic Toxicity Data and Proposed Testing for Members of the Polybutylene Succinic Anhydride Category

CAS Number	ACUTE TOXICITY TO FISH 96-hr LC_{50} (mg/L)	ACUTE TOXICITY TO INVERTEBRATES 96-hr LC_{50} (mg/L)	TOXICITY TO ALGAE 96-hr EC_{50} (mg/L)
	Available Data & Proposed Testing	**Available Data & Proposed Testing**	**Available Data & Proposed Testing**
67762-77-0	LL_{50} >1000 mg/L (WAF, RT)	EC_{50} >1000 mg/L (WAF, DM)	EC_{50} >1000 mg/L (WAF, PK)
67762-79-2	No testing needed Bridging	No testing needed Bridging	No testing needed Bridging

WAF = Water Accommodated Fraction
RT = Rainbow Trout
DM = *Daphnia magna*
PK = *Pseudokirchneriella subcapitata*

Table 6. Acute Mammalian Toxicity Data for Members of the Polybutylene Succinic Anhydride Category

CAS Number	ACUTE ORAL TOXICITY[1]	ACUTE DERMAL TOXICITY[1]
	Available Data	Available Data
67762-77-0	LD_{50} > 2.0 g/kg (rat)	LD_{50} > 2.0 g/kg (rat)
67762-79-2	LD_{50} > 2.0 g/kg (rat)	LD_{50} > 2.0 g/kg (rabbit)

[1]Toxicity endpoints are expressed as median lethal dose (LD_{50}) for acute oral and dermal toxicity.

Table 7. Mutagenicity Data and Proposed Testing for Members of the Polybutylene Succinic Anhydride Category

CAS Number	GENE MUTATION ASSAY	CHROMOSOMAL ABERRATION ASSAY
	Available Data & Proposed Testing	Available Data & Proposed Testing
67762-77-0	Bacterial Reverse Mutation Assay - Not mutagenic	Test
67762-79-2	Bacterial Reverse Mutation Assay - Not mutagenic	No testing needed Bridging

Table 8. Repeated-dose Mammalian Toxicity Data and Proposed Testing for Members of the Polybutylene Succinic Anhydride Category

CAS Number	REPEATED-DOSE TOXICITY	REPRODUCTIVE/DEVELOPMENTAL TOXICITY
	Available Data & Proposed Testing	Available Data & Proposed Testing
67762-77-0	Technical Discussion*	Technical Discussion*
67762-79-2	Technical Discussion*	Technical Discussion*

* Technical discussion will be developed pending test results of structurally similar - see Alkenyl Succinic Anhydride Category (Group 16).

CAS Number	Environmental Fate					Ecotoxicity			Human Health Effects				
	Physical Chem	Photo deg	Hydro lysis	Fugacity	Bio deg	Acute Fish Tox	Acute Invert Tox	Alga Tox	Acute Tox	Point Mutations	Chrom Effects	Subchronic	Repro/ Develop
67762-77-0	C/T	D/C	L(T)	C	A	A	A	A	A	A	T	R/D	R/D
67762-79-2	C	D/C	D	C	B	B	B	B	A	A	B	R/D	R/D

A Adequate data available
B Bridging data from another category member
C Computer modeling proposed
D Technical discussion proposed
T Test
R Read-across from a structurally similar material
L Literature review (testing will be conducted if inadequate information is found in the literature review)

AR201-14084B

Substance Group: Group 27

Summary Prepared by: Petroleum Additives Panel
Health & Environmental Research Task Group

Date of last update: November 26, 2002

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1.0 Biodegradation

Robust Summary 27-BioDeg-1

Test Substance	
CAS #	67762-77-0
Chemical Name	2,5-Furandione, dihydro-, monopolyisobutylene derivatives
Remarks	Test material purity not provided
Method	
Method/Guideline Followed	OECD 301B, Ready Biodegradability, Modified Sturm Test
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (study performed)	1995
Contact time (units)	28 days
Test apparatus	Six glass 4-liter Erlenmeyer flasks containing two liters of modified biochemical oxygen demand water (BOD).
Inoculum	Activated sewage sludge from a domestic wastewater treatment plant, prepared per test guideline. Inoculum was not acclimated. Twenty milliliters of inoculum added to all test flasks.
Replicates:	Duplicates for test substance and blank controls, single flasks for the reference substance and toxicity control.
Temperature of incubation:	21.6-23.4 °C
Dosing procedure:	Neat test chemical was gravimetrically determined on glass cover slips, which were then added to culture medium in test vessels.
Study initiation:	Test flasks provided with CO_2 free air and placed on a rotary shaker and mixed at 110rpm for the study duration.
Sampling:	Days 2, 4, 7, 10, 14, 17, 21, 24, 29 (after acidification on day 28)
Concentration of test substance:	10 mg C/L weighed directly onto tared glass slides and placed into each test substance flask.
Controls:	Toxicity, blank and positive controls used per guideline. Positive control was benzoic acid (Na salt) added to the control vessel at a loading of 10 mg C/L. Both test substance and reference material were added to the toxicity control flask to obtain a maximum concentration of 20 mg C/L
Analytical method:	Titration of residual Ba(OH)2 (0.05 N initially) in trapping solution, using 0.05N HCl.
Study termination:	The pH of the content of each test flask was determined. The flasks were then acidified with 1 ml of concentrated sulfuric acid to drive off inorganic carbonate. A sample was centrifuged and a subsample of the concentrate was submitted for soluble organic carbon (SOC) analysis.
Method of calculating biodegradation values:	Percent biodegradation calculated as percent ratio of cumulative net carbon dioxide to theoretical carbon dioxide as determined from elemental analysis of test material.

Results	The test substance was not considered readily biodegradable under the criteria that requires 60% biodegradation within 28 days, achieved within 10 days of reaching 10% biodegradation. The CO_2 production from the reference chemical exceeded the 60% of theoretical necessary to consider the test valid. The % TCO_2 for the toxicity control was > 25 as of day 12 therefore the test substance was not considered to be inhibitory at the concentration tested.
Degradation % After Time	Test substance: 2.3-5.0 % TCO_2 in 28days Positive control substance: 88.1% in 28 days
Conclusions	The test substance was not readily biodegradable (2.3-5.0% TCO_2 in 29 days).
Data Quality	(1) Reliable without restriction
References	This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information).
Other	Updated: 11/13/2002

2. 0 Acute Toxicity to Fish

Robust Summary 27-FISH-1

Test Substance	
CAS #	67762-77-0
Chemical Name	2,5-Furandione, dihydro-, monopolyisobutylene derivatives
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD 203
Test Type	Acute Toxicity to Fish (Water Accommodated Fraction-WAF)
GLP (Y/N)	Y
Year (Study Performed)	1996
Species/Strain	Rainbow Trout (*Oncorhynchus mykiss*)
Fish Number	20/concentration (10/exposure chamber, duplicate chambers/concentration; 0.83 grams of fish/liter)
Fish Size	Average length 50.3 mm; Average weight 1.25 g
Analytical Monitoring	Yes (Total Organic Carbon determinations)
Nominal Test Substance Concentration Levels	0, 130, 220, 370, 600 and 1000 mg/l
Test Concentration Preparation	Test solutions were prepared separately for each replicate test concentration by adding an appropriate aliquot (by weight) of test material to 35 liters of dilution water in glass vessels. The solutions were stirred vigorously for approximately 20 hours. Following a settling period of 4 hours the water accommodated fraction (WAF) of each replicate test concentration was siphoned into each replicate test vessel.
Exposure Period	96 hours
Exposure Conditions	Static-renewal test conditions. At 24, 48 and 72 hours of exposure the test fish were carefully transferred into aquaria containing fresh test solutions.
Vehicle	None
Statistical Analysis	None required based on the results.
Dose Rangefinding Study	Yes
Test Chambers	20-liter glass aquaria containing 15 liters of test solution
Diluent Water	Deionized water
Diluent Water Chemistry	Hardness 40-48 mg/l as $CaCO_3$ Alkalinity 39 mg/l as $CaCO_3$ Conductivity 130 umhos/cm pH 7.5-7.8
Diluent Water Chemistry During 96 Hour Exposure Period.	Dissolved Oxygen: 7-9.8 mg/L pH: 6.5-8.1 Conductivity: 120-160 umhos/cm
Photoperiod	16 hours of light, 8 hours of dark
Temperature Range	11-13 ^{O}C during 14 day holding period 11-12 ^{O}C during exposure period
Remarks field for test conditions	All organisms were observed for mortality and the number of individuals exhibiting clinical signs of toxicity or abnormal behavior at 2, 24, 48, 72, and 96 hours after initiation of test material exposure.

Results	Analysis of freshly prepared and 24 hour control and test solutions for total organic carbon resulted in the following results: Nominal WAF Concentration (mg/L) / TOC (mg/L) 0 hr / TOC (mg/L) 24 hr 0 (control) 2.1-2.2 3.2-4.2 130 2.8 2.7-3.3 1000 4.8-5.0 6.2-6.5 Throughout the test period a film of dissolved test material was observed on the surface of test solutions at a concentrations of 220 mg/L WAF and higher. WAFs at a concentration of 370 mg/L and higher were slightly cloudy for at least a portion of the 96 hour test period. Insoluble test material was not noted in any other test solution. Fish in the negative control group were normal throughout the study. No mortality was observed at 130, 220 or 600 mg/L. At 370 mg/L 10% mortality was observed at 96 hours. At 1000 mg/L 15% and 25% mortality were observed at 72 and 96 hours respectively. At 48 and 72 hours some fish at 1000 mg/L appeared lethargic and exhibited a loss of equilibrium. The mortality and other effects observed at higher loading rates may have been due to physical effects of undissolved test material (e.g. fouling of gills). The 24, 48, 72 and 96-hour LC50s were each greater than 1000 mg/L (WAF). The 96 hour no observed effect level was 600 mg/L.
Conclusions	Under the conditions of this study the 24, 48, 72 and 96-hour LC50s were each greater than 1000 mg/L (WAF). The 96 hour no observed effect level was 600 mg/L.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 11/13/2002

3.0 Acute Toxicity to Aquatic Invertebrates (e.g. Daphnia)

Robust Summary 27-DAPH-1

Test Substance	
CAS #	67762-77-0
Chemical Name	2,5-Furandione, dihydro-, monopolyisobutylene derivatives
Remarks	Purity not provided
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1300 (1987), OECD Guideline for Testing of Chemicals #202 *Daphnia* sp. Acute Immobilization Test and Reproduction Test (1984).
Test Type	Static acute toxicity test
GLP (Y/N)	Y
Year (Study Performed)	1996
Species/Strain	*Daphnia magna*
Analytical Monitoring	Total organic carbon (TOC) measurements of initial (0-h) test solutions and at 48 hours post initiation of exposure.
Exposure Period (unit)	48 hours
Statistical methods	Based on 100% survival no statistical analysis was required.
Remarks field for test conditions (fill as applicable)	Juvenile daphnids less than 24-hours old were produced from laboratory in-house culture. Individual water accommodated fractions (WAFs) were prepared for each test level and renewed daily. A measured weight of test material was added to a measured volume of dilution water (1.5 L) in a glass vessel and stirred for 20 hours. Stirring was accomplished using a Teflon coated magnetic stir bar. Following the mixing period, the test solutions were allowed to stand for 4 hours before the water phase was gently siphoned from the mixing vessel into corresponding replicate test vessels (1 liter/vessel). The toxicity test was conducted in 300 ml glass beakers that contained 250 ml of test solution. Thirty daphnids, less than 24 hours old were distributed into each concentration (10 daphnids/replicate) within 30 minutes of test solution preparation. At 24 hours the test solutions were replaced with newly prepared WAF and all surviving daphnids were carefully transferred into the corresponding test vessel. Daphnids were not fed during exposure. Control test chambers/daphnids were handled in an identical fashion. Light cycles were maintained at 16-hour light per day with an intensity of 7 uEin/m^2sec. Test solutions were maintained at 20 $\pm$ 1 degree C. Dilution water was filtered well water adjusted to the appropriate hardness of 160-180 mg/L as $CaCO_3$.
Test Concentrations	0 and 1000 mg/L WAF
RangeFinding Study	Yes; concentration range of 10 to 1000 mg/L WAF
Results	24 and 48-h EC_{50} >1,000 mg/L (WAF). 48 hour NOEC is 1000 mg/L.
Remarks	Water chemistry: Dissolved oxygen: 7.7-8.5 mg/L; pH: 7.5 - 8.0; conductivity: 560 – 600 umhos/cm. Total Organic Carbon measurements were 6.4 mg/L and 2.2mg/L in the 1000 mg/L test concentration solution and in the control solution at

	48 hours. Analysis of 0 hour test solutions resulted in measurements of 1.2mg/L and 6.4mg/L in the control and 1000 mg/L test concentration solution. No insoluble test material was noted during the study. Following 48 hours of exposure, no dead or immobilized organisms were observed at the treatment level (1000 mg/L). . The 48-hour EC50 was determined to be greater than 1000 mg/L, the only concentration tested. The no observed effect concentration was established as 1000 mg/L.
Conclusions	The 24 and 48-h EC_{50} >1,000 mg/L (WAF). The no observed effect concentration was established as 1000 mg/L.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 10/28/2002

4.0 Acute Toxicity to Aquatic Plants (e.g. algae)

Robust Summary 27-ALG-1

Test Substance	
CAS #	67762-77-0
Chemical Name	2,5-Furandione, dihydro-, monopolyisobutylene derivatives
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1050 (1993), OECD Guideline for Testing of Chemicals #201 Alga, Growth Inhibition Test (1984).
Test Type	Static acute toxicity test
GLP (Y/N)	Y
Year (Study Performed)	1996
Species/Strain	Freshwater algae, *Pseudokirchneriella subcapitata* formerly called *Selenastrum capricornutum*
Element basis (# of cells/mL)	Approximately 10,000 cells/mL
Exposure period/duration	96 hours
Analytical monitoring	Total organic carbon (TOC) measurements of initial (0-h) and final (96-h) control and test solutions followed EPA Method 415.1 (unfiltered).
Statistical methods	The 24, 48, 72 and 96 hour effective concentrations (EC10, EC50, EC90) could not be calculated using standard statistical techniques because cell growth at the single tested concentration equaled 90 to 100 % of the cell growth of the controls throughout the test. The 96 hour NOEC was determined using TOXSTAT 3.3, which calculated a t-test, comparing the growth of algae of the controls to the growth of algae in the treated group.
Remarks field for test conditions (fill as applicable)	Test Species: Cells taken from an in-house culture of *Pseudokirchneriella subcapitata* that was originally purchased from the University of Texas at Austin alga collection. Test System: The WAF was prepared only at the beginning of the test. A measured weight of test material was added to a measured volume of dilution water (1-L) in a glass vessel and stirred for 20 hours. Stirring accomplished using a magnetic stirrer. Mixing speed was adjusted such that a vortex formed approximately 25% of the distance to the bottom. Following the mixing period, the test solution was allowed to stand for 4 hour before the water phase was removed. The siphoned water phase (i.e., WAF) was used for the aquatic toxicity test. Test Conditions: A static test was conducted; i.e., there was no daily renewal of test solution. Three 100-mL replicates per treatment, inoculum ~10,000 cells/mL. The 250-mL Erlenmeyer flasks were covered to reduce entry of dust, etc. During the test all treatment and control flasks were randomly placed on an orbital shaker adjusted to approximately 100 cycles per minute under constant light (24 hours/day). Daily cell counts, the occurrence of relative size differences, unusual cell shapes, colors, flocculations, adherence of

	cells to test containers or aggregation of cells was determined visually by means of direct microscopic examination with a hemocytometer. Light: Cool-white fluorescent lights provided a light intensity of approximately 400 foot-candles. Test temperature: 24.0 C. Dilution Water: Sterile enriched alga growth media (US EPA, 1978) adjusted to pH 7.5. Measured TOC and total suspended solids in fresh untreated alga media were <1.0 and <10 mg/L, respectively. Test media pH was 7.4 at 0-hour and 9.6 - 10.6 after 96 hours. Test Levels: Control and 1,000 mg/L WAF loading rates. No undissolved test material was seen on the surface of the test vessels during the entire aquatic toxicity test. Method of calculating mean measured concentrations: not applicable Exposure period: 96 hours Analytical monitoring: At the beginning and end of the test, TOC levels were non-detect (<1) – 2.1 mg/L in control and 5.8 – 6.7 mg/L at 1,000 mg/L. TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates.
Results	96-h EC_{50} >1,000 mg/L; The 96-hr NOEC = 1,000 mg/L.
Remarks	Test Findings: At 96-hours biomass measurement in the treated group was 95% of the control at 1,000mg/L (WAF). Control response was satisfactory.
Conclusions	The test material was not toxic to freshwater alga at a loading rate of 1,000 mg/L. 96-h EC_{50} >1,000 mg/L; The 96-hr NOEC = 1,000 mg/L.
Data Quality	(1) Reliable without restriction
References	Confidential business information.
Other	Updated: 11/13/2002

5.0 Acute Oral Toxicity

Robust Summary 27-AcuteOral-1

Test Substance	
CAS #	CAS# 67762-77-0
Chemical Name	2,5-Furandione, dihydro-, monopolyisobutylene derivatives
Remarks	Test material dosed as received, purity not provided.
Method	
Method/Guideline followed	OECD Guideline 401
Test Type	Acute oral toxicity
GLP (Y/N)	Y
Year (Study Performed)	1996
Species/Strain	Rats/Sprague-Dawley
Sex	Male/Female
No. of animals/dose	5 /sex/group
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	5000 mg/kg
Dose volume	5.56 ml/kg
Control group	Yes
Chemical analysis of dosing solution	No
Remarks field for test conditions	A single dose of the undiluted test material was administered intragastrically to five fasted male and female rats. Clinical observations were conducted at 1, 2.5, and 4 hours after test material administration and daily thereafter for 14 days. The animals were observed for mortality twice daily. Individual body weights were recorded on the day of dosing, on day 7 and at termination. All animals were euthanized, and gross necropsies were performed, at the conclusion of the observation period.
Results	LD50 > 5 g/kg
Remarks	There were no deaths during the study. All animals exhibited body weight gains during the study. All animals appeared normal throughout the study. There were no significant necropsy findings evident in the surviving animals.
Conclusions	The test article, when administered to 5 male and 5 female rats had an acute oral LD50 of > 5 g/kg. No significant toxicity was observed.
Data Quality	Reliable without restriction (Klimisch Code).
References	Unpublished confidential business information
Other	Updated: 8/27/01

Robust Summary 27-AcuteOral-2

Test Substance	
CAS #	CAS# 67762-79-2
Chemical Name	2,5-furandione, dihydro-,monopolybutenyl derivatives
Remarks	Test material dosed as received, purity not provided.
Method	
Method/Guideline followed	FHSA 16CFR1500.3
Test Type	Acute oral toxicity
GLP (Y/N)	N
Year (Study Performed)	1978
Species/Strain	Rats/ Wistar strain
Sex	Male
No. of animals/dose	10
Vehicle	Mazola Corn Oil (50% mixture)
Route of administration	Oral (intragastric)
Dose level	5, 7.12, 10.14 and 14.43 g/kg
Dose volume	Not provided
Control group included	No
Remarks field for test conditions	A single dose of the test material was administered intragastrically to 10 fasted (over night) male rats at each treatment level. A control group was not included. The animals were observed for signs of toxicity or behavioral changes 3-4 hours after dosing and daily for 14 days. Individual weights were recorded on the day of dosing and at termination. All animals were euthanized at the conclusion of the observation period. Gross autopsies were performed on all animals after 14 days.
Results	LD50 >14.9 g/kg (males)
Remarks	All of the animals survived the study. At 14.43 g/kg diarrhea, chromorhinorrhea and oily bodies were noted in 5 or more animals. Isolated instances of ptosis, chromodacryorrhea and piloerection were also noted. At 10.14 g/kg diarrhea, chromorhinorrhea, piloerection, ptosis and lethargy were noted in 5 or more animals. Isolated instances of ataxia and chromodacryorrhea were also noted. At 7.12 g/kg isolated instances of diarrhea, piloerection, lethargy and respiratory noise were noted. At 5.0 g/kg isolated instances of diarrhea and chromorhinorrhea were noted. All animals gained body weight during the study. Gross necropsy findings were unremarkable for all animals.
Conclusions	The test article, when administered to male Wistar rats, had an acute oral LD50 of >14.9 g/kg.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 1/21/02

6.0 Acute Dermal Toxicity

Robust Summary 27-Acute Dermal-1

Test Substance	
CAS #	CAS# 67762-77-0
Chemical Name	2,5-Furandione, dihydro-, monopolyisobutylene derivatives
Remarks	Test material dosed as received, purity not provided.
Method	
Method/Guideline followed	OECD Guideline 402
Test Type	Acute dermal toxicity (Limit Test)
GLP (Y/N)	Y
Year (Study Performed)	1996
Species/Strain	Rats/Crl:CD®(SD)BR
Sex	Male and female
No. of animals/sex	5
Vehicle	None
Route of administration	Dermal
Dose level	2 g/kg (0.014 g/cm^2)
Application area	Approximately 36 cm^2
Control group included	No
Remarks field for test conditions	Prior to the initiation of dosing the back and flanks of each animal were clipped of hair to expose 20% of the total body surface. Animals were reclipped as needed. A single dose of 2 g/kg of the undiluted test material was administered dermally to five male and female rats. The test material was kept in contact with the skin for a period of 24 consecutive hours, on approximately 20% of the total body surface under a semi-occlusive bandage that was loosely over wrapped with a sheet of perforated plastic film. At the end of the 24-hour exposure period, the application site was wiped clean of residual test material with mineral oil, followed by liquid Ivory soap mixed with warm tap water, rinsed with tap water, and dried with a paper towel. The animals were observed for abnormal clinical signs at 1, 2.5, and 4 hours after dosing and daily for the 14-day study period. Dermal examinations were performed 30 minutes post test material removal and on days 3, 7, 10 and 14 according to the Draize method. Individual body weights were recorded on day 1, prior to dosing, and on days 7 and 14. The surviving animals were euthanized at the conclusion of the observation period. Gross necropsies were performed on all animals.
Results	LD50 > 2.0 g/kg (males and females)
Remarks	No mortality was observed. Clinical observations were unremarkable. All animals exhibited body weight gains during the study. A slight erythema and edema reaction was observed in one male (days 1 and 3). Slight erythema was observed in one female (day 1). These findings cleared by day 7 in the male and by day 3 in the female. There were no macroscopic findings associated with treatment.

Conclusions	The test article, when administered dermally as received to 5 male and 5 female Sprague-Dawley rats, had an acute dermal LD50 of greater than 2.0 g/kg. Evidence of slight dermal irritation was observed in two animals during the first week of study.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 8/3/01

Robust Summary 27-AcuteDermal-2

Test Substance	
CAS #	CAS# 67762-79-2
Chemical Name	2,5-furandione, dihydro-,monopolybutenyl derivatives
Remarks	Test material dosed as received, purity not provided.
Method	
Method/Guideline followed	Similar to OECD Guideline 402
Test Type	Acute dermal toxicity (Limit Test)
GLP (Y/N)	N
Year (Study Performed)	1978
Species/Strain	Rabbits/New Zealand White
Sex	Male
No. of animals/group	4
Vehicle	None
Route of administration	Dermal
Dose level	20 g/kg
Dose volume	Not provided.
Control group included	Yes
Remarks field for test conditions	This study was conducted prior to the development of Test Guideline 402. This study deviated from Guideline 402 in that the skin of 2 of 4 treated animals was abraded prior to dosing. In addition the guideline calls for the evaluation of males and females using at least one dose level. This study was conducted using males only. These deviations were not considered sufficient to change the outcome of the study. Immediately prior to topical application of the test material, the abdominal hair of each animal was closely clipped. The skin of two of the four treated animals was abraded prior to test material administration. A single dose of 20 g/kg of the undiluted test material was administered dermally to four male animals. The test material was kept in contact with the skin for a period of 24 consecutive hours under a gauze bandage covered with an elastic sheet. The application site was wiped clean of residual test material at the end of the 24-hour exposure period. The animals were observed for 14 days after treatment. Irritation was scored (Draize) on day 1 post treatment. All animals were euthanized at the conclusion of the observation period. Gross necropsies were not performed.
Results	LD50 > 20.0 g/kg (males)
Remarks	All animals survived the duration of the study. Lethargy, diarrhea, bloated abdomen and difficulty walking due to soreness of the exposure area were noted in 2 or more animals. Isolated instances of mucus in the stool were also noted. Slight to well defined erythema and very slight-to-slight edema were noted in the treated skin of all animals at 24 hours post dosing. Body weight data was unremarkable.

Conclusions	The test article, when administered dermally as received to 4 male New Zealand white rabbits had an acute dermal LD50 of greater than 20.0 g/kg (males)
Data Quality	Reliable without restriction (Klimisch Code).
References	Unpublished confidential business information
Other	Updated: 1/21/02

7. 0 Gene Mutation Assays

Robust Summary 27-GenTox-1

Test Substance	
CAS #	CAS# 67762-77-0
Chemical Name	2,5-Furandione, dihydro-, monopolyisobutylene derivatives
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD Guideline 471
Test Type	Bacterial Reverse Mutation Assay
GLP (Y/N)	Y
Year (Study Performed)	1996
Test System	*Salmonella typhimurium* and *Escherichia Coli*
Strains Tested	*Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537; *Escherichia Coli* tester strain WP2*uvr*A
Exposure Method	Plate incorporation
Test Substance Doses/concentration levels	Initial assay: *Salmonella* + (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate *Salmonella* - (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate WP2*uvr*A + (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate WP2*uvr*A - (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate Confirmatory assay: *Salmonella* + (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate *Salmonella* - (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate WP2*uvr*A + (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate WP2*uvr*A - (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate
Metabolic Activation	With and without (500 ul of 10% S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats)
Vehicle	Pluronic F127 (25% w/w in ethanol)
Vehicle Control	Pluronic F127 (25% w/w in ethanol)
Tester strain, activation status, Positive Controls and concentration level	TA98 +S9 benzo(a)pyrene 2.5 ug/plate TA98 -S9 2-nitroflourene 1.0 ug/plate TA100 +S9 2-aminoanthracene 2.5 ug/plate TA100 -S9 sodium azide 2.0 ug/plate TA1535 +S9 2-aminoanthracene 2.5 ug/plate TA1535 -S9 sodium azide 2.0 ug/plate TA1537 +S9 2-aminoanthracene 2.5 ug/plate TA1537 -S9 ICR-191 2.0 ug/plate WP2*uvr*A +S9 2-aminoanthracene 25.0 ug/plate WP2*uvr*A –S9 4-nitroquinoline-N-oxide 1.0 ug/plate
Statistical Analysis	Mean revertant colony count and standard deviation were determined for each dose point.
Dose Rangefinding Study	Conducted using tester strains TA100 and WP2*uvr*A and ten doses of test material ranging from 10.0 to 10,000 ug/plate, one plate/dose with (10% S9 homogenate/ml of S9 mix) and without metabolic activation. Cytotoxicity was evaluated.
S9 Optimization Study	Conducted using tester strains TA98 and TA100, and a non-cytotoxic dose level of test article (5000 ug/plate) and four concentrations of S9 mix (5, 10, 20 and 80% S9 homogenate/ml of S9 mix). Cytotoxicity was evaluated.

Remarks field for test conditions	In the main study there were two treatment sets for each tester strain, with (+S9) and without (-S9) metabolic activation. Each of the tester strains was dosed with six concentrations of test substance, vehicle controls, and a positive control. Three plates/dose group/strain/treatment set were evaluated. The results of the initial assay were confirmed in a second independent experiment. 50 ul of test material, positive control or vehicle control were added to each plate along with 100 ul of tester strain, S9 mix (if needed) and 2.0 (with S9) or 2.5 ml (without S9) of top agar. This was overlaid onto the surface of 25 ml minimal bottom agar in a petri dish. Plates were incubated for 48 hours at 37^{o}C. Plates that were not evaluated immediately were held at 5^{o}C until evaluated. The condition of the bacterial background lawn was evaluated for cytotoxicity and test article precipitate. The number of revertant colonies/plate for the vehicle controls and all plates containing test article were counted manually. The number of revertant colonies/plate for the positive controls were counted by automated colony counter.
Results	The test substance was not genotoxic in this assay with or without metabolic activation.
Remarks	In the dose rangefinding study no cytotoxicity was observed with tester strain TA100 or WP2*uvr*A at dose levels up to 10,000 ug/plate with or without metabolic activation. Test article precipitate was observed on plates at 667 ug/plate and above with tester strain TA100 with activation and at 1000 ug/plate and above without activation. With WP2*uvr*A without metabolic activation precipitate was observed at 667 ug/plate and above. With activation, with WP2*uvr*A, precipitate was observed at 333 ug/plate and above. Based on these results the dose levels outlined above (page 1, Test Substance Doses, Initial Assay) were selected. The S9 optimization study was performed using TA98 and TA100 with a non-cytotoxic dose of test article, (5000 ug/plate) and four concentrations of S9 mix (5, 10, 20 and 80% S9 homogenate/ml of S9 mix). In the absence of any effect a 10% S9 mix was used in the mutagenicity study. In the initial assay all data were acceptable and a dose responsive increase in the mean number of revertants/plate was observed with tester strain TA100 only in the absence of activation. In addition non-dose responsive increases were observed with TA98 and WP2*uvr*A with activation. These results were unexpected in light of the range find work and the S-9 optimization study. For these reasons the confirmatory assays were conducted using the same dose levels. In these confirmatory mutagenicity assays all data were acceptable and no positive increases in the number of revertants/plate were observed with any of the tester strains with or without metabolic activation. Based on these results all tester strains were retested for confirmation again. In these confirmatory mutagenicity assays all data were acceptable and no positive increases in the number of revertants/plate were observed. Based on these results the test material was considered not mutagenic. The initial positive results seen in three tester strains were considered spurious.

	No cytotoxicity was observed up to 10,000 ug/plate with the *Salmonella* tester strains with and without activation and with WP2*uvr*A with and without activation. Test material participate was observed on plates at ≥250 ug/plate. The positive control for each respective test strain exhibited at least a 3-fold increase (with or without S9) over the mean value of the vehicle control for a given strain, confirming the expected positive control response.
Conclusions	Under the conditions of this study, the test material was not mutagenic.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 8/6/01

Robust Summary 27-GeneTox-2 --- MORE THAN ONE STUDY AVAILABLE!!

Test Substance	
CAS #	CAS# 67762-79-2
Chemical Name	2,5-furandione, dihydro-,monopolybutenyl derivatives
Remarks	77% Active Ingredient
Method	
Method/Guideline followed	OECD Guideline 471
Test Type	Bacterial Reverse Mutation Assay
GLP (Y/N)	N
Year (Study Performed)	1979
Test System	*Salmonella typhimurium*
Strains Tested	*Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537, TA1538
Exposure Method	Plate incorporation
Test Substance Doses/concentration levels	0.33, 1.0, 3.33, 10.0 and 33.3 ul/plate
Metabolic Activation	With and without (0.5 ml of S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats)
Vehicle	Ethanol
Tester strain, activation status, Positive Controls and concentration level	TA98 +S9 Aflatoxin B1 1.0 ug/plate TA98 -S9 2-nitroflourene 0.5 ug/plate TA100 +S9 Aflatoxin B1 1.0 ug/plate TA100 -S9 N-methyl-N-nitro-N-nitrosoguanidine 5.0 ug/plate TA1535 +S9 2-aminoanthracene 2.5 ug/plate TA1535 -S9 N-methyl-N-nitro-N-nitrosoguanidine 5.0 ug/plate TA1537 +S9 2-aminoanthracene 2.5 ug/plate TA1537 -S9 9-aminoacridine 100 ug/plate TA1538 +S9 2-aminofluorene 2.0 ug/plate TA1538 -S9 2-nitroflourene 5.0 ug/plate
Vehicle Control	Ethanol 100 ul/plate
Statistical Analysis	Mean revertant colony count and standard deviation were determined for each dose point. Linear regression analysis was used to compute the best-fit line of dose response.
Dose Rangefinding Study	No
S9 Optimization Study	Yes
Remarks field for test conditions	This study was conducted in 1979, prior to the adoption of OECD Test Guideline 471. In addition to the tester strains used during this study, the OECD Guideline suggests the inclusion of tester strains *E.coli* WP2 uvrA, or WP2 uvrA (pKM101) or *Salmonella typhimurium* TA102. This study included the use of tester strain TA1538. OECD 471 does not incorporate this strain. These deviations from the test guideline are not considered major study deficiencies. In the main study there were two treatment sets for each tester strain, with (+S9) and without (-S9) metabolic activation. Each of the tester strains was dosed with five concentrations of test substance, vehicle control, and a positive control. Three plates/dose group/strain/treatment set were evaluated. Test material, positive control or vehicle control were added to each plate along with 0.1 ml of tester strain, and S9 mix (if needed). This was overlaid onto the

	surface of supplemented Noble's agar in a screw-capped tube. Tubes were mixed and poured over a base plate of Spizzizen's minimal medium. Plates were incubated for 48 hours at 37°C.
Results	The test substance was not genotoxic in this assay with or without metabolic activation.
Remarks	In this mutagenicity assay all data were acceptable and no positive increases in the number of revertants/plate were observed with any of the tester strains with or without metabolic activation. The positive control for each respective test strain exhibited at least a 3-fold increase (with or without S9) over the mean value of the vehicle control for a given strain, confirming the expected positive control response. For each strain, the numbers of revertant colonies in negative control plates were within acceptable limits as defined by historical control data for spontaneous revertants. Sterility controls were negative.
Conclusions	Under the conditions of this study, the test material was not mutagenic.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 11/13/01